

Potential of Entomopathogenic nematodes versus alpha-cypermethrin against Potato Tuber Moth, *Phthorimaea operculella* Zeller 1873 (Lep.: Gelechiidae) in storage conditions

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Abstract

The potato tuber moth PTM, *Phthorimaea operculella*, is one of the most economically important potato pests worldwide. In the present study, the potential of *Steinernema feltiae* and *Steinernema carpocapsae* for controlling PTM in potato tubers was evaluated compared to alpha-cypermethrin. *Steinernema carpocapsae* in both concentrations (12.6×10^6 IJs and 6.2×10^6 IJs) showed a lower number of emerged insects than alpha-cypermethrin (10 mg ai l^{-1}). Alpha-cypermethrin (20 mg ai l^{-1}) showed the highest efficacy against PTM (81.17%), *S. carpocapsae* (12.6×10^6 IJs), and alpha-cypermethrin (10 mg ai l^{-1}) showed similar efficacy (72.53%) while *S. feltiae* (6.2×10^6 IJs) showed the lowest efficacy (39.04%). The results showed that *S. carpocapsae* in both concentrations and *S. feltiae* (12.6×10^6 IJs) were efficient the same as alpha-cypermethrin (10 mg ai l^{-1}) having no environmental and health adverse impacts issued in the chemical insecticides usage. Both concentrations of alpha-cypermethrin and *S. carpocapsae* showed the least tuber damage with no significant differences, while it was as high as the control (59.26%) in both concentrations of *S. feltiae*. This promising finding introduces EPNs as a part of the potato tuber protection program in storage. Accordingly, EPNs can be considered as an appropriate alternative to synthetic chemicals for PTM control without any residue and health problems.

Keywords: EPNs, PTM, biological control, potato tuber, efficacy, alpha-cypermethrin

1. Introduction

Potatoes play a critical role in feeding the human population and sustainable potato production is currently a serious challenge facing agricultural experts worldwide (Vincent et al. 2013; Devaux et al. 2014; Kroschel et al. 2020). The potato tuber moth PTM, *Phthorimaea operculella* Zeller 1873 (Lep.: Gelechiidae), is an oligophagous invasive pest of Solanaceae family (Sileshi and Teriessa 2001) and one of the most economically significant potato pests worldwide which causing losses up to 80% in the field and up to 100% during storage especially in tuber infestation (Rondon 2010, 2020; Gallego et al. 2020). Assessment of the effect of temperature increase under projected changes in global temperature for the year 2050 by process-based climatic response phenology models demonstrated that the damage potential of *P. operculella* will progressively increase in all regions where the pest exist, with a range expansion into temperate and tropical mountainous regions (Kroschel et al. 2013, 2016). Due to Rondon (2020), foliar damage of PTM to the potato crop does not usually result in significant yield losses. In contrast, tuber infestation reduces the yield heavily both in the farm and the storage. Therefore, control of PTM in tubers is critical.

Traditional control of *P. operculella* has relied upon chemical insecticides (Szendrei 1986; Kroschel et al. 2020), however as a leaf miner or tuber miner, *P. operculella* cannot touch many insecticides directly (Gao & Zhou 2020). Even though there are serious health and environment-related concerns about using chemical insecticide (Kroschel & Koch 1996; Lacey & Kroschel 2009; Gallego et al. 2020), they are the main part of PTM management programs.

Entomopathogenic nematodes (EPNs) currently available commercially for biological control of insects, are classified into two families, Heterorhabditidae and Steinernematidae. They have some advantages as biocontrol agents, including actively searching the host, which is a critical trait in cryptic habitant and miner insect pests (Grewal & Georgis 1999; Shapiro-Ilan et

al. 2012). Therefore, EPNs are prime candidates for biological control of tuber moth larvae (Wraight et al. 2007), and results of some laboratory and field research conducted on the effect of EPNs against PTM revealed their good potential (Ivanova et al. 1994; Lacey & Kroschel 2009; Yan et al. 2020).

Most of the research on EPNs effects against PTM was carried out on the PTM larval stages out of the tubers. In the present study potential of *Steinernema feltiae* and *Steinernema carpocapsae* for controlling PTM in potato tubers and their effects on the tuber damage reduction was evaluated, which is a more practical application method. Also, due to the usage of chemical insecticides as the primary PTM control approach, the efficacy of EPNs was compared with alpha-cypermethrin. Higher or equal efficacy of EPNs in comparison with alpha-cypermethrin is promising result.

2. Materials and Methods

2.1. Insects

The stock culture of the PTM population was reared on potato tubers and maintained at room temperature of 25 ± 3 °C, 16 h light: 8 h dark, and $60 \pm 5\%$ relative humidity. Potato tubers were used for PTM rearing. The plexiglass-case cages (35 cm × 25 cm × 10 cm) with the lid sealed with a fine mesh cloth were used. The bottom of the cage was covered with a thin layer of autoclaved fine sand for pupation. The tubers were washed and air-dried, then used as oviposition sites and food sources. For infesting a tuber with more than one neonate larva, the tubers were punctured with a fine needle. Then the tubers and the same age eggs were added to the cages. For collecting the same-age eggs, daily collected pupa from sieving the sand of the bottom of the rearing cage were added to a cylindrical plastic container (8 cm × 13 cm; 750 ml) as a mating cage. The container was covered with fine mesh gauze. A source of feeding (10%

sucrose solution) and a piece of filter paper (Whatman No. 1) wetted with sterile water were placed on the mesh gauze to provide an oviposition site for the moths. PTM does not require a host plant's stimulus to initiate mating and egg-laying, unlike some phytophagous insects (Fenemore 1978). The filter paper was replaced each 24 h and kept at 25 °C for 48h. The eggs were used for the tuber infestation in the experiments.

2.2. Nematodes

Commercial products of *S. carpocapsae* (Capsanem[®]) and *S. feltiae* (Entonem[®]) were provided from Koppert Biological Systems (Berkel en Rodenrijs, The Netherlands).

2.3. Chemical Insecticide

Alpha-cypermethrin 100 SC (Shef[®], Pirakeshtshimi company, Iran) was provided. It is classified in group 3A of IRACas as a pyrethroid insecticide.

2.4. Efficacy of The EPNs and Alpha-cypermethrin Against PTM

Desired concentrations of both *S.feltiae* and *S. carpocapsae* were made using distilled water. Five samples were taken from the final nematode suspensions and alive infective juveniles (IJs) were counted for survival assessing the products. The final concentrations used in the experiments were 12.6×10^6 IJs l⁻¹ and 6.2×10^6 IJs l⁻¹ for both EPN species. The nematode suspension was shaken continuously, and 20 to 25 medium-sized potato tubers (100 to 150 g weight) were immersed in the nematode suspensions for 30 seconds. The tubers were then taken out and instantly after dripping the excess water. The tubers were placed into the straw paper boxes (56 cm × 21 cm × 36 cm). PTM eggs immediately before hatching were added to the treated tubers. For the infestation of each 40 tubers, 1500 eggs were used. Lid of the boxes were sealed with fine mesh cloth. The treated tubers were placed at three levels of the boxes (floor, 15 cm higher than the floor, 30 cm higher than the floor). The boxes were filled with insect-free

washed and dried potato tubers. Two alpha-cypermethrin concentrations (20 mg ai l⁻¹ and 10 mg ai l⁻¹) were used for the tuber treatment. The tuber immersing method was used as described for EPNs assay. The experiment was repeated three times for each nematode species and alpha-cypermethrin. Distilled water was used as control. The boxes were kept at 25±3 °C and dark conditions. The boxes were monitored daily for emerging pupae or adults after three weeks. When the adults were observed, number of the emerged insects in treatments, the number of the insects inside the tubers, and the damage percentage for each tuber were recorded.

2.5. Data Analysis

The corrected efficacy percentage was calculated according to Henderson and Tilton formula (Henderson and Tilton 1955). Analysis of variance was carried out using SAS software, and Duncan's multiple range test, was estimated to compare the means (SAS 2012).

3. Results

3.1. Number of Different Stages of PTM In the Treated Potato Tubers

All treatments including both nematode species and alpha-cypermethrin in both concentrations showed lower number of PTM compared with control (F= 30.93; df=6, 42; P<0.0001; Table 1); however, the level of the boxes showed no significant effect on total number of PTM (F=2.15; df= 2, 42; P=0.128; Table 1). Among the treatments, *S. feltiae* (6.2×10⁶IJs) showed the highest total number of PTM followed by *S. feltiae* (12.6×10⁶IJs), alpha-cypermethrin (10 mg ai l⁻¹) *S. carpocapsae* (12.6×10⁶IJs), *S. carpocapsae* (6.2×10⁶IJs), and alpha-cypermethrin (20 mg ai l⁻¹), which showed the lowest total number of PTM (Figure 1). In the control and all the treatments, pupae were made the largest part of the population (Figure 1). Due to the results, *S. carpocapsae* in both concentrations were more efficient than alpha-cypermethrin (10 mg ai l⁻¹), which is

promising. The number of adult PTM was the highest at 15 cm above the floor and the lowest at the floor (Figure 2).

3.2. EPNs and Alpha-cypermethrin Efficacy Against PTM

The efficacy of the treatments were significantly different ($F=28.01$; $df= 5, 36$; $P<0.0001$; Table 2). The effect of the boxes' level was significant on the treatment efficacy ($F=29.12$; $df=2, 36$; $P<0.0001$; Table 2) while the interaction of the treatments and the levels had no significant differences (Table 2). Mean comparison for the efficacy of the treatments against PTM resulted in developing three different groups, including alpha-cypermethrin (20 mg ai l^{-1}) with the highest efficacy, *S.carpocapsae* (12.6×10^6 IJs), alpha-cypermethrin (10 mg ai l^{-1}), *S.carpocapsae* (6.2×10^6 IJs), and *S.feltiae* (12.6×10^6 IJs) with similar efficacy and *S.feltiae* (6.2×10^6 IJs) with the lowest efficacy (Figure 3). Therefore the results showed that *S.carpocapsae* in both concentrations and *S.feltiae* (12.6×10^6 IJs) were as efficient as alpha-cypermethrin (10 mg ai l^{-1}) while they have no environmental and health concern issues related to the chemical insecticides usage. The effect of the level on the efficacy of EPNs and alpha-cypermethrin did not show a regular and interpretable pattern. The treatments' efficacy was the highest on the floor, followed by 30 cm above the floor and 15 cm above the floor (Figure 4).

3.3. Potato Tubers Damage Percentage

The data reflecting the damage percentage of the potato tubers treated with EPNs and alpha-cypermethrin are summarized in Table 2. Analysis of variance for the potato tubers damage showed significant differences among the treatments ($F=21.38$; $df=6, 42$; $P<0.0001$), also, the level of the boxes had a significant effect on the damage percentages ($F= 9.38$; $df=2, 42$; $P=0.0004$). Comparing the levels showed that the third level (30 cm above the floor) had the

highest damage. In contrast, the first and second levels showed no significant differences (Figure 4). According to the results, the treatments' interaction and the levels were significant ($F=3.17$; $df=12, 42$; $p=0.002$).

The damage of PTM on the tubers in control was 59.26%, followed by *S. feltiae* (both concentrations) with no significant difference. In contrast, both concentrations of *S. carpocapsae* and alpha-cypermethrin similarly showed lower damage percentage (Figure 3).

4. Discussion

Entomopathogenic nematodes are obligate parasites of insects used for biological control of economically important insect pests (Shapiro-Ilan et al. 2012). Their relatively high efficacy against PTM was reported previously (Ivanova et al. 1994; Lacey & Kroschel 2009; Yan et al. 2020; Mhatre et al. 2020); most of these researches were conducted on the pupa and larval stages of PTM in Petri Dish. However, Ivanova et al. (1994) reported the high efficacy of different species of EPNs against PTM on potato tubers. They sprayed the PTM infested potato tubers with aqueous suspensions of *S. feltiae* and *S. carpocapsae* at the rate of 20000 IJs. They observed 95.5 and 93.1% PTM larval mortality, respectively. In the present study, *S. feltiae* (12.6×10^6 IJs⁻¹) and both concentrations of *S. carpocapsae* showed high efficacy as much as alfa-cypermethrin (10 mg ai l⁻¹), and this is a unique point. However, tuber damage was higher in both concentrations of *S. feltiae* with no significant differences compared to the control. Many factors are responsible for the efficacy of EPNs against targeted hosts, including tolerance of environmental conditions (Lacey & Georgis 2012; Shapiro-Ilan et al. 2012), the pathogenicity of the nematode and symbiotic bacteria complex against the targeted host (Hasan et al. 2019; Heryanto & Eleftherianos 2020), nematode host finding strategy (Grewal et al. 2005),

application rate (Jagdale et al. 2004), means of application and agrochemicals (Shapiro-Ilan et al. 2012; Griffin 2012). In the present study, two EPN species that were used showed some differences in efficacy and reducing potato tuber damage. Overall, both species were compatible with alpha-cypermethrin at least at 10 mg ai l⁻¹ concentration; however, *S. carpocapsae* was a little superior to *S. feltiae*. These two species have relatively remarkable differences in their host finding strategy, species of the symbiotic bacteria, and the natural host insect range. *Steinernema carpocapsae* is known as an ambusher species with a sit and wait foraging strategy, which is the most effective in the soil surface, cryptic habitats, and hosts with high mobility, while *S. feltiae* is a cruiser one with a mobile foraging strategy which is most effective against sedentary hosts in foliar, epigeal and cryptic habitats (Campbell et al. 2003; Lewis et al. 2006; Lacey & Georgis 2012). PTM larvae are sedentary hosts in a cryptic habitat inside the potato tubers. Based on the foraging strategy definition, *S. feltiae* should be more successful than *S. carpocapsae* in finding and infecting PTM. However, recently Wilson et al. (2012) hypothesized that due to the adaptation of *S. carpocapsae* to habitats other than mineral soils, including peat, bark, or wood and leaf litter, this species is capable of cruising long distances toward the hosts. Therefore, *S. carpocapsae* is an ambusher, yet it behaves equally as a cruiser in such organic habitats. This idea was suggested before by Kruitbos et al. (2010) and the capability of *S. carpocapsae* in the control of some sedentary hosts in cryptic habitats (Lacey & Unruh 1998; Martinez de Altube et al. 2008; Ennis et al. 2010) confirms this hypothesis. The results of the present study showed that *S. carpocapsae* behaved as a cruiser nematode and infected the host inside the potato tubers successfully.

Xenorhabdus nematophila and *X. bovienii* are symbiont of *S. carpocapsae* and *S. feltiae*, respectively. Symbiotic bacteria have a critical role in the efficacy of EPNs against their host

species; probably, they are partially responsible for pathogenicity and host range of EPNs. In terms of host range, *S. carpocapsae* have a more comprehensive natural host range and reported from more than 10 families from four insect orders, especially lepidopteran and coleopteran insects, while *S. feltiae* has been found to infect a naturally narrower range of the insects in Coleoptera (Scarabaeidae, Curculionidae) and Lepidoptera, mainly Noctuidae and most frequently Diptera (Peters 1996; Poinar 1992). Consequently, *S. feltiae* is more effective against dipteran insects. Its commercial products are used against economic dipteran insects especially fungus gnats (Gouge & Hague 1995; Georgis et al. 2006). Therefore, the superiority of *S. carpocapsae* in lepidopteran pest control is expected.

5. Conclusion

The results showed that *S. carpocapsae* in both concentrations (6.2×10^6 and 12.6×10^6 IJs) could reduce PTM damage on the potato tubers as well as alpha-cypermethrin (10 and 20 mg ai l⁻¹). This promising finding introduces EPNs as a part of the potato tuber protection program in storage. Accordingly, EPNs can be considered as an appropriate alternative of the synthetic chemicals for PTM control with no residue and health concern issues.

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Table1: Analysis of variance for larva, pupa, adult, and the total number of PTM in different treatments

Source of Variance	Df	Mean Square			
		Larva	Pupa	Adult	Total number
Treatment	6	51.67**	7793.21**	1521.26**	14403.3**
Block	2	3.05	121.52	221.71	134.02
Level	2	3.59 ^{ns}	60.30 ^{ns}	223.28**	134.40 ^{ns}
Treatment × Level	12	2.83 ^{ns}	447.42**	119.47**	522.24**
Error	40	1.60	31.70	4.16	60.39

^{ns} and **: non-significant and significant at 1% probability level, respectively.

Table 2: Analysis of variance for the treatments efficacy against PTM and potato tuber damage percentage

Source of Variance	Efficacy			Damage %		
	Df	Mean square	F Value	Df	Mean square	F Value
Treatment	5	1823.68**	30.39	6	1058.91**	23.08
Block	2	151.7	2.53	2	122.68	2.67
Level	2	1895.76**	31.59	2	464.52**	10.13
Treatment × Level	10	105.11 ^{ns}	1.75	12	156.95**	3.42
Error	36	65.10		40	49.53	

ns , * and **: non-significant and significant at 5% and 1% probability level, respectively.

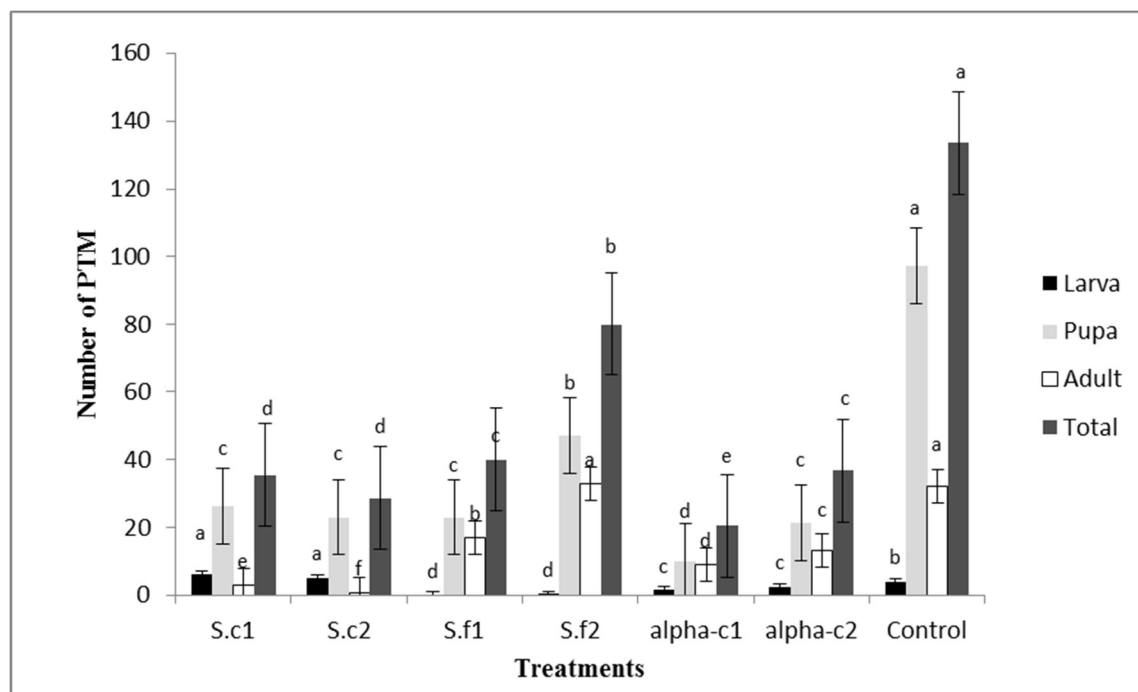


Fig. 1

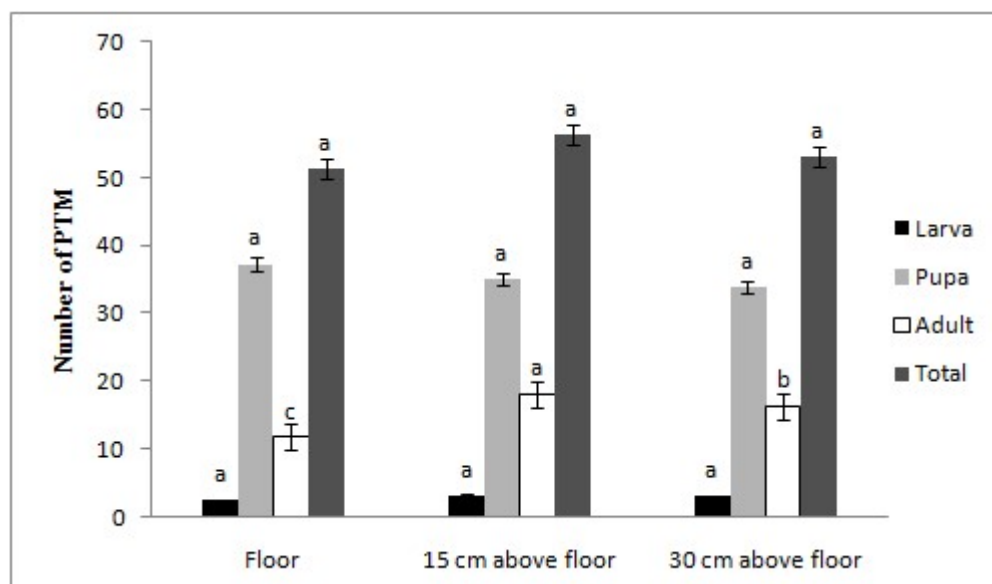


Fig. 2

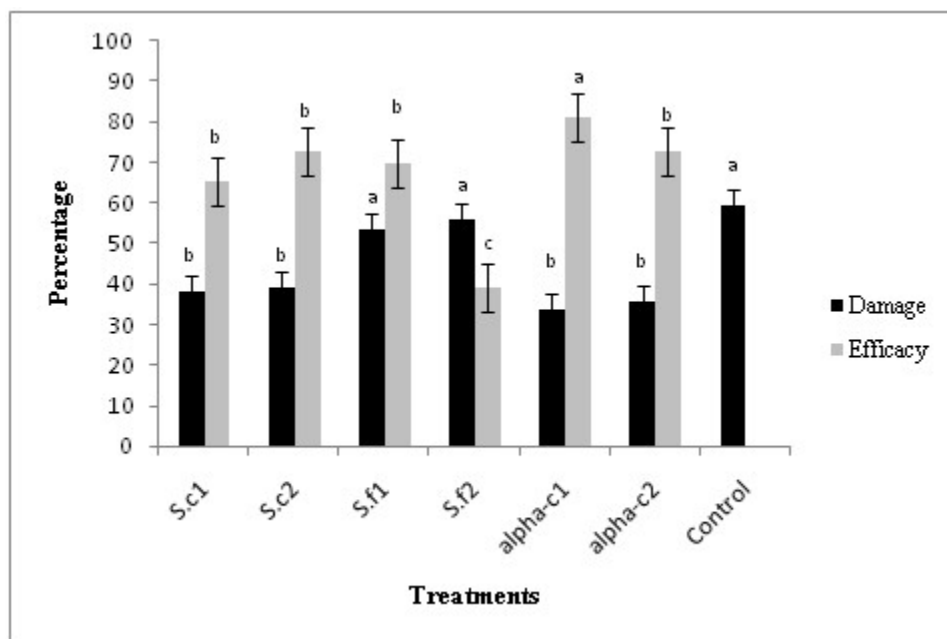


Fig. 3

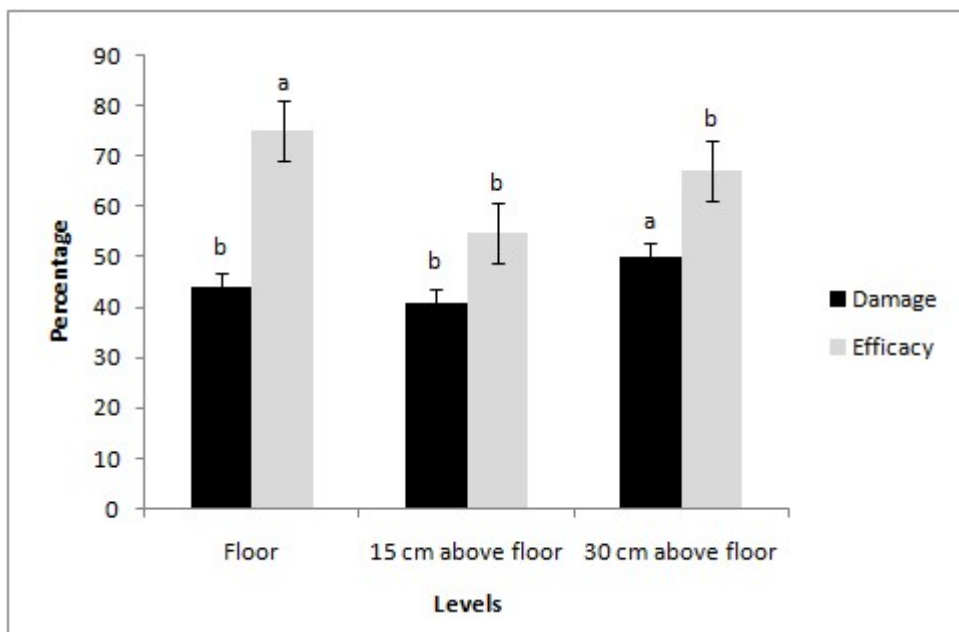


Fig. 4

Figure captions

Fig. 1 Number of the emerged PTM from treated potato tubers in the treatments: *S. carpocapsae* (12.6×10^6 IJs), (S.c1); *S. carpocapsae* (6.2×10^6 IJs), (S.c2); *S. feltiae* (12.6×10^6 IJs), (S.f1); *S. feltiae* (6.2×10^6 IJs), (S.f2); alpha-cypermethrin (20 mg ai l^{-1}), (alpha-c1); alpha-cypermethrin (10 mg ai l^{-1}), (alpha-c2); untreated (control). Different letters on bars indicate significant differences among the PTM life stages in different treatments according to Duncan's multiple range test ($p < 0.05$)

Fig. 2 Number of the emerged PTM from treated potato tubers in different levels. Different letters on bars indicate significant differences among life stages in different levels according to Duncan's multiple range test ($p < 0.05$)

Fig. 3 Efficacy of different treatments against PTM and damage percentage of PTM on treated potato tubers; *S. carpocapsae* (12.6×10^6 IJs), (S.c1); *S. carpocapsae* (6.2×10^6 IJs), (S.c2); *S. feltiae* (12.6×10^6 IJs), (S.f1); *S. feltiae* (6.2×10^6 IJs), (S.f2); alpha-cypermethrin (20 mg ai l^{-1}), (alpha-c1); alpha-cypermethrin (10 mg ai l^{-1}), (alpha-c2); untreated (control). Different letters on bars indicate significant differences according to Duncan's multiple range test ($p < 0.05$)

Fig. 4 Efficacy of different treatments against PTM and damage percentage of PTM on treated potato tubers at different levels. Different letters on bars indicate significant differences according to Duncan's multiple range test ($p < 0.05$)